J. Clin. Chem. Clin. Biochem.
Vol. 15, 1977, pp. 359–360

SHORT COMMUNICATION

Demonstration of a Collagenolytic Enzyme in Postmortal Human Colon Tissue

By *H. Kortmann* and *S. von Bary*

Chirurgische Klinik (Direktor: Prof. Dr. G. Heberer) der Universität München

(Received October 5, 1976/January 20, 1977)

Summary: A collagenolytic enzyme was extracted from the 6000 *g* sediment of homogenized postmortal human colon tissue. The enzyme digests native collagen at neutral pH. The enzyme has been concentrated by ammonium sulfate precipitation. The collagenolytic activity was measured using [1-^{14}C]acetylated calf skin collagen, solubilized in 0.1 g/l acetic acid. There was a significant inactivation of the enzyme solution after the addition of 6 µmol EDTA to the test volume.

Nachweis eines kollagenspaltenden Enzyms in postmortalem menschlichen Dickdarmgewebe

Zusammenfassung: Aus dem 6000 *g* Sediment von homogenisiertem menschlichem Dickdarmgewebe wurde ein Enzym extrahiert, das natives Kollagen bei neutralem pH spaltet. Die Extraktion erfolgte mit einem neutralen Tris/HCl Puffer, der 1 mol/l NaCl enthielt. Durch Ammoniumsulfatfällung wurde das Enzym konzentriert. Die kollagenspaltende Aktivität wurde mit [1-^{14}C]-acetyliertem Kalbshautkollagen als Substrat, das in 0,1 g/l Essigsäure gelöst war, bestimmt. Durch Zugabe von 6 µmol EDTA zum Testansatz konnte die kollagenspaltende Aktivität der enzymhaltigen Lösung gehemmt werden.

Introduction

In the last two decades collagenases were isolated from various mammalian tissue cultures (1, 2). Since their possible involvement in the postoperative breakdown of colonic and rectal anastomoses had often been emphasized (3, 4, 5), we examined extracts from the 6000 *g* sediment of postmortal human colon tissue for collagenolytic activity.

Material and Methods

Colon tissue

Postmortal human colon preparates were received from the Institute of Pathology of the University of Munich. Immediately after delivery the preparates were frozen in liquid nitrogen and stored at – 70 °C. Only colon sections without any pathological impairment were used. The postmortal period before freezing was limited to 24 h.

The thawed colon was washed with cold water. The mucosa and submucosa were separated and collected (1280 g wet weight in total). The tissue (1 g) was homogenized in 10 volumes (10 ml) of ice-cold 0.05 mol/l Tris/HCl buffer, pH 7.6 containing 10 mmol/l $CaCl_2$, 0.25 mol/l sucrose, and 0.2 g/l NaN_3 using an Ultra-Turrax homogenizer.

Extraction

The homogenates were centrifuged at 6000 *g* for 30 min at 4 °C. The sediment was suspended in 0.05 mol/l Tris/HCl buffer, pH 7.6 containing 10 mmol/l $CaCl_2$, 1 mol/l NaCl, 0.2 g/l NaN_3 and stirred for extraction over a period of 48 h at 4 °C. Afterwards the suspension was centrifuged again at 40 000 *g* at 4 °C. The supernatant was used for the enzyme assay. Portions of the supernatant were treated with ammonium sulfate in order to get precipitates at 0.20, 0.40, 0.60, 0.80, and 1.00 saturation at 4 °C. The pellets were solubilized in Tris/HCl buffer and after dialysis against the same buffer measured for collagenolytic activity. Protein concentration was determined by the Biuret method (6).

Preparation of the ^{14}C-acetylated collagen

The ^{14}C-acetylated collagen was prepared according to the method of *Gisslow & McBride* (7). A solution of 60 mg of acid-soluble calf skin collagen in 10 ml 0.075 mol/l sodium citrate, pH 3.7 (EGA Chemie, Steinheim/Albuch) was added to 200 ml of 0.1 g/l acetic acid and ultrafiltrated in an Amicon cell (membrane UM-2) to a volume of 30 ml. Immediately prior to addition of the acetylating agent, the pH of the collagen solution was adjusted to 8.9 by addition of 1 mol/l K_2HPO_4. The acetylating agent, 2.4 mg [1-^{14}C]acetic anhydride in 0.1 ml benzene water (20 g + 80 g) (specific activity 8.7 MBq/mg = 238 µCi/mg; purchased from Amersham Buchler GmbH & Co KG) was added dropwise to the reaction mixture over a period of 30 min. During this process the temperature was kept at 10 °C and the pH at 8 by addition of 1 mol/l NaOH. After the anhydride had been added the mixture was further stirred for 1 h. The pH of the mixture was then adjusted to 4.0 by addition of glacial acetic acid. The solution of ^{14}C-acetylated collagen was dialysed against water for 3 days until ^{14}C was no longer traceable in the dialysates. The solution was lyophilized and the ^{14}C-acetylated collagen stored at – 20 °C. The final preparation had a specific activity of about 1.5×10^5 counts/min · mg collagen. Reaction mixtures containing 400 µg ^{14}C-acetylated collagen and 50 µg trypsin[1]) (2 U/mg; E. Merck, Darmstadt) up to a volume of 500 µl never exceeded more than 5% of the radioactivity released by the enzyme-free blanks.

Assay of collagenolytic activity

^{14}C-labeled collagen was solubilized in 0.1 g/l acetic acid by stirring for about 12 h at 4 °C. The reaction mixture contained 200 µl (400 µg) ^{14}C-acetylated collagen; 200 µl 0.1 mol/l Tris/HCl buffer, pH 7.2 containing 5 mmol/l $CaCl_2$, and 0.2 g/l NaN_3. After gel formation at 37 °C in a water bath 100 µl of the test solution were added. The inhibitory effects of EDTA (E. Merck, Darmstadt) and bovine pancreatic trypsin inhibitor (5700 KIE[2]/mg; Bayer AG, Leverkusen) were tested by addition of 10 µl of a 0.6 mol/l EDTA-solution, pH 7.2, or 50 µg bovine pancreatic trypsin inhibitor to the mixture. The reaction mixture was incubated at 37 °C for 3, 6, 14, and 24 h. After incubation the reaction mixtures were filtered through 0.8 µm pore size, 13 mm diameter, MF-Millipore filter (Millipore GmbH, Neu-Isenburg). The filter retained undigested fibrils (8). 100 µl of the filtrate was removed and ^{14}C-activity was measured in a Packard-Tri-Carb Liquid Scintillation Spectrometer Model 3003. The values were corrected by subtracting the counts obtained with an enzyme-free control tube. The total activity per assay tube was 6400 counts/min. Collagenolytic activity was expressed as counts/min of the filtrate.

[1]) EC 3.4.21.4.
[2]) KIE = Kallikrein Inhibiting Unit.

Tab. 1. Collagenolytic activity of extracts from the 6000 *g* sediment of postmortal human colon tissue. BPTI: bovine pancreatic trypsin inhibitor.

	Incubation time 37 °C [h]	Radioactivity per tube released [counts/min]	Protein of extract per tube [μg]	Release of total activity per tube [%]	Specific activity after 24 h [counts/min·μg protein]	Inhibition [%]
Total radioactivity per assay tube	24	6399	–	100	–	–
Trypsin control 50 μg/tube	24	254	–	4	–	–
Extracts from colon tissue	3	203	289	3.2	–	–
	6	528	289	8.3	–	–
	14	1045	289	16.3	–	–
	24	3003	289	47	10.4	–
+ 6 μmol/tube EDTA	24	848	289	13	2.9	72
+ 50 μg/tube BPTI	24	2998	289	47	10.4	< 1

Results and Discussion

Table 1 shows the collagenolytic activities found in extracts from the 6000 *g* sediment of human colon homogenates after different incubation times. When the extracts were treated with ammonium sulfate nearly all the activity could be found in the precipitates obtained between 0.20 and 0.60 saturation. Beyond 0.60 saturation no further activity was detectable in the supernatants.

Most mammalian collagenolytic enzymes have been shown by cell culture techniques. The present experiments demonstrate the presence of a collagenolytic enzyme in the mucosa and submucosa of postmortal human colon. However, before extraction of the 6000 *g* sediment with Tris/HCl buffered NaCl-solution no significant collagenolytic activity could be measured in the homogenates. This fact may be explained by a tightly bound tissue enzyme, as already shown for some mammalian collagenases (9, 10, 11), or by high affinity inhibitors. There was no inhibition of the collagenolytic activity by the bovine pancreatic trypsin inhibitor but we found a 72% inactivation of the enzyme after the addition of EDTA to the test volume. Further studies will show whether there are further similarities with known mammalian collagenases.

Acknowledgements

The authors would like to thank Prof. Dr. *H. Fritz*, Institut für Klinische Chemie und Klinische Biochemie and Prof. Dr. *W. Brendel*, Institut für Chirurgische Forschung an der Chirurgischen Klinik der Universität München, for enabling them to carry out this study. Financial support was granted by Prof. Dr. *H. Fritz*.

References

1. Harris, E. D. & Krane, S. M. (1974), N. Engl. J. Med. *291*, 557–563, 605–612, 652–659.
2. Wooley, D. E., Tucker, J. S., Green, G. & Evanson, J. M. (1976), Biochem. J. *153*, 119–126.
3. Hunt, T. K. (1975), Current Surgical Diagnosis & Treatment (Dunphy, J. E. & Way, L. W. eds) p. 102, Lange Medical Publications, Los Altos, California.
4. Delaney, P; & Lalor, D. (1976), Br. J. Surg. *63*, 23–24.
5. Hawley, P. R., Faulk, W. P., Hunt, T. K. & Dunphy, J. E. (1970), Br. J. Surg. *57*, 869–900.
6. Bergmeyer, H. U., Bernt, E., Gawehn, K. & Michal, G. (1974), Methoden der enzymatischen Analyse (Bergmeyer, H. U. ed.) Bd. 1, 184–185 Verlag Chemie, Weinheim/Bergstr.
7. Gisslow, M. T. & McBride, B. C. (1975), Anal. Biochem. *68*, 70–78.
8. Robertson, P. B.; Taylor, R. E. & Fullmer, H. M. (1972), Clin. Chim. Acta *42*, 43–45.
9. Montfort, I. & Pérez-Tamayo, R. (1975), Conn. Tiss. Res. *3*, 245–252.
10. Wirl, G. (1975), Hoppe-Seyler's Z. Physiol. Chem. *356*, 1289–1295.
11. McCroskery, P. A., Rochards, J. F. & Harris, E. D. (1975), Biochem. J. *152*, 131–142.

Dr. med. Helmut Kortmann und
Dr. med. Siegfried von Bary
Chirurgische Klinik der Universität München
Nußbaumstraße 20
D-8000 München 2
West-Germany